09/710,444
pg 39 spec

Table 8. Selection of catalytically active phage-Stoffel particles.

φ_i [a] in tu	φ_f [b] in tu	Yield in %	Conditions [c]
$8.4x10^5$	$2.0x10^4$	2.4	
$3.6x10^5$	$1.0x10^2$	0.028	- primer 1b
$4.4x10^5$	$3.0x10^2$	0.068	- biotinylated dUTP 2
$4.8x10^5$	$3.0x10^2$	0.062	- template 3
$4.4x10^9$	$4.0x10^6$	0.091	- trypsin
$1.5x10^9$	$5.5x10^5$	0.037	- trypsin, - primer 1b

φ_i and φ_f denote the number of transformation units (tu) prior [a] and after [b] the selection. Yield = $\varphi f / \varphi i$.

[c]: + primer **1b**, + biotinylated dUTP **2**, + template **3** and + trypsin.

Example 9. Selection for disulphide-containing polypeptides.

For the cloning of (poly)-peptide encoding DNA fragments and their display for selection between barnase and p3, the phage fd-3 is constructed (Fig. 5). Phage fd-3 comprises the H102A mutant of barnase N-terminally fused to the p3 gene of phage fd-TET. Between the codon for the last residue of barnase and the first residue of p3 is the nucleotide sequence *CTG CAG* GCG GTG CGG CCG CA. This sequence contains a PstI DNA restriction site (in italics) for insertion of DNA fragments flanked by PstI restriction sites. The sequence further introduces a frame shift between barnase and p3, which prevents expression of the correct p3 reading frame in fd-3. Phage particles of phage fd-3 therefore do not display the infection protein p3 and are non-infectious.

see 7/30/02 for correction to add seq ID NO: 1

Phage fd-3 is therefore well suited as a cloning vector as vectors without PstI DNA inserts after ligation are not propagated during selection. Statistically 1 out of 3 random DNA inserts in the PstI restriction site will (except for the presence of stop-codons